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Poor Outcome in Disseminated Intravascular Coagulation or Thrombotic Thrombocytopenic Purpura Patients With Severe Vascular Endothelial Cell Injuries

Hideo Wada,[1]* Yoshitaka Mori,[2] Minori Shimura,[1] Katzuyo Hiyoyama,[1] Mika Ioka,[1] Takahiro Nakasaki,[1] Masakatsu Nishikawa,[1] Masahiko Nakano,[3] Kousuke Kumeda,[4] Toshihiro Kaneko,[1] Shin Nakamura,[5] and Hiroshi Shiku[1]

[1]Second Department of Internal Medicine, Mie University School of Medicine, Tsu-city, Japan
[2]Mie Red Cross Blood Center, Tsu-city, Japan
[3]Mitubishi Gas Chemical, Niigata-city, Japan
[4]Chemo-Sero-Therapeutic Research Institute, Kumamoto-city, Japan
[5]Department of Cellular and Molecular Biology, Primate Research Institute, Kyoto University, Inuyama-city, Japan

Various hemostatic and vascular endothelial cell markers were measured in patients with disseminated intravascular coagulation (DIC), non-DIC, or thrombotic thrombocytopenic purpura (TTP) and in healthy volunteers to examine the relationships between the hemostatic abnormalities or vascular endothelial cell injuries and the patients' outcomes. Although the plasma levels of soluble fibrin monomer, thrombin-antithrombin complex, plasmin-plasmin inhibitor complex, and D-dimer were significantly increased in the DIC patients, there were no significant differences in these markers between the DIC patients who survived and those who died, suggesting that these markers might not be directly related to the patient outcome. The plasma thrombomodulin (TM) levels in the DIC and TTP patients were significantly higher than those in the healthy volunteers, and the plasma TM levels in the patients who died were significantly higher than those in the patients who survived. These findings showed that the TM level reflected the outcome, and that the outcome of the diseases underlying DIC and TTP might depend on vascular endothelial cell injuries. The plasma protein C and antithrombin activities were markedly reduced in the DIC, non-DIC, and TTP patients who died compared to those who survived. These findings suggest that reduced plasma antithrombin and protein C activities are useful markers of systemic vascular endothelial injuries. Although the plasma tissue factor (TF) levels were significantly increased in the DIC patients, there was no significant difference in the plasma TF levels between the DIC patients who died and those who survived. In conclusion, we found that the outcome of the diseases underlying DIC and TTP is related to vascular endothelial cells, and that plasma TM, antithrombin, and protein C are useful markers for systemic vascular endothelial cell injury. Am. J. Hematol. 58: 189–194, 1998. © 1998 Wiley-Liss, Inc.

Key words: thrombomodulin; TTP; DIC; outcome of systemic vascular endothelial cell injuries

INTRODUCTION

Disseminated intravascular coagulation (DIC) is associated with severe bleeding tendency and organ failure, and its outcome is poor [1,2]. Severe hypercoagulability is observed at the onset of DIC, although the markers for hypercoagulability, i.e., thrombin-antithrombin complex (TAT), D-dimer, and soluble fibrin monomer (SFM), are not related to the outcome of patients with DIC [3]. Thrombotic thrombocytopenic purpura (TTP) [4,5] is characterized by fluctuating bizarre neurological symptoms, consumptive thrombocytopenia, microangiopathic hemolytic anemia, renal dysfunction, and fever. More than 20% of TTP patients treated with plasma exchange do not survive [6]. The plasma thrombomodulin (TM),

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*Correspondence to: Hideo Wada, M.D., Second Department of Internal Medicine, Mie University School of Medicine, 2-174 Edobashi, Tsu-city, Mie-ken 514, Japan.

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TABLE I. Underlying Diseases of DIC and Non-DIC*

	DIC		Non-DIC	
	Survived	Deaths	Survived	Deaths
Hematopoietic malignancies	5	7	13	5
Solid cancers	4	4	13	2
Infections	2	3	6	2
Aneurysm	3	1	0	1
Others	1	0	7	2
Total	15	15	39	12

*DIC, disseminated intravascular coagulation.

TABLE II. Numbers of Patients Surviving and Deceased at 4 Weeks After the Blood Sampling*

	Survived	Deaths	Total
DIC	15	15	30
Non-DIC	38	12	50
TTP	13	7	20
HUS	9	0	9
Total	74	34	108

*DIC, disseminated intravascular coagulation; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome.

tissue type plasminogen activator (t-PA), and plasminogen activator inhibitor-I (PAI-I) levels were found to be significantly increased in patients with DIC [7] and those with TTP [8], suggesting that vascular endothelial cell injuries exist in these patients. The organ failure associated with systemic vascular endothelial cell injury in these diseases is a poor prognostic factor.

We measured various hemostatic factors and vascular endothelial cell markers in DIC patients, non-DIC patients, and TTP patients and examined the relationships between the hemostatic abnormalities, vascular endothelial cell injuries, and the patients' outcomes.

MATERIALS AND METHODS

We examined 30 patients with DIC, 50 patients without DIC (non-DIC), 20 patients with TTP, 9 patients with hemolytic uremic syndrome (HUS), and 30 healthy volunteers. The diseases underlying the DIC and those of the non-DIC patients were as follows: hematopoietic malignancy, 30 patients; solid cancer, 23 patients; infections, 13 patients; aneurysm, 5 patients; and other disease, 10 patients (Table I). The hemostatic parameters of these patients were followed up during their clinical course at the Second Department of Internal Medicine, Mie University School of Medicine. The diagnosis of DIC was based on the criteria established by the Japanese Ministry of Health and Welfare [9,10]. The DIC patients were treated with gabexate mesilate (FOY), a synthetic proteinase inhibitor [11] that inhibits the activity of thrombin, factor Xa, plasmin, and plasma kallikrein. The non-DIC patients were treated only for their underlying diseases. Fifteen of the 30 patients with DIC died within 4 weeks of blood sampling, and 12 of the 50 non-DIC patients died within 4 weeks of blood sampling. In all of the DIC and non-DIC patients, the plasma creatinine level was less than 2.0 mg/dl.

The patients who had severe thrombocytopenia, schistocytic hemolytic anemia, fluctuating neurological signs, and negative verotoxin were diagnosed as having TTP, and the patients who had severe thrombocytopenia, schistocytic hemolytic anemia, and positive verotoxin were diagnosed as having HUS. The TTP patients were treated with a plasma exchange, antiplatelet agents, and steroids, and hemodialysis was performed in the severe HUS patients. Seven of the 20 patients with TTP died within 4 weeks of the blood sampling. None of the 9 patients with HUS died within 4 weeks of blood sampling (Table II). Blood samples were obtained on admission or at the onset of the disease.

The methods used for measuring the activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen were described previously [12]. Antithrombin activity and protein C activity were measured by an amidolytic assay using a Berichrom-Antithrombin kit (Behringwerke AG, Marburg, Germany) and a Berichrom-Protein C kit (Behringwerke AG), respectively. The TAT, plasmin-plasmin inhibitor complex (PPIC), D-dimer, and TM levels were measured by enzyme-linked immunoassays (ELISA) using Enzygnost TAT (Behringwerke AG), PPIC test (Teijin, Tokyo, Japan), Frelisa D-dimer (Agen, Brisbane, Australia), and a thrombomodulin enzyme immunoassay (EIA) kit (Mitsubishi Gas Chemical, Tokyo, Japan). The SFM level was measured by ELISA using the Enzymun test (Behringwerke AG) [13]. Plasma tissue factor (TF) antigen was measured by a TF ELISA kit (Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan).

The results are expressed as the mean ± one standard deviation (SD). Statistical significance was calculated by the nonparametric Mann-Whitney U-test. Probability values less than 0.05 were considered significant.

RESULTS

APTT and PT were prolonged in the patients with DIC, and the plasma levels of SFM, TAT, PPIC, D-dimer, and FDP were significantly increased in the DIC patients. Although the PT was slightly prolonged in the DIC patients who died, there was no significant difference in the plasma fibrinogen, SFM, TAT, PPIC, D-dimer, or FDP between the DIC patients who survived and those who died. The plasma levels of SFM, TAT, PPIC, D-dimer, and FDP were also slightly increased in the non-DIC patients and TTP/HUS patients. The plasma levels of SFM and D-dimer were slightly increased in the

TABLE III. Hemostatic Data of the DIC, Non-DIC, and TTP/HUS Patients*

		DIC		Non-DIC		TTP/HUS		
		Survived	Deaths	Survived	Deaths	Survived	Deaths	Normal
APTT	(sec)	40.0 ± 16.5	57.1 ± 28.8	31.6 ± 4.9	37.9 ± 11.3	37.8 ± 9.5	42.4 ± 4.2	31.7 ± 5.1
PT	(sec)	14.9 ± 2.5	19.9 ± 9.7	12.7 ± 1.4	13.2 ± 1.0	12.5 ± 0.9	12.8 ± 1.6	11.8 ± 2.3
Fibrinogen	(μg/dl)	188 ± 91	183 ± 121	303 ± 91	274 ± 96	248 ± 68	210 ± 68	252 ± 39
SFM	(μg/ml)	320 ± 140	319 ± 160	43.2 ± 56.8	140 ± 151	34.8 ± 19.7	59.2 ± 19.0	0.3 ± 0.1
TAT	(ng/ml)	30.9 ± 29.1	51.4 ± 55.3	14.6 ± 14.5	17.4 ± 21.4	13.9 ± 9.4	31.9 ± 24.3	1.8 ± 0.7
PPIC	(μg/ml)	6.7 ± 6.1	5.03 ± 4.63	1.39 ± 1.21	1.69 ± 1.38	1.23 ± 0.71	1.74 ± 1.25	0.4 ± 0.2
D-dimer	(μg/ml)	24.7 ± 15.6	30.9 ± 27.0	3.6 ± 5.5	11.2 ± 9.1	3.1 ± 3.9	5.5 ± 2.0	0.2 ± 0.1
FDP	(μg/ml)	42.3 ± 10.4	47.8 ± 25.0	13.7 ± 8.4	15.2 ± 7.5	11.5 ± 7.2	14.6 ± 8.9	2.8 ± 2.7

* Data are mean ± SD. DIC, disseminated intravascular coagulation; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; APTT, activated partial thromboplastin time; PT, prothrombin time, SFM, soluble fibrin monomer; TAT, thrombin-antithrombin complex; PPIC, plasmin-plasmin inhibitor complex.

non-DIC patients who died, and the plasma SFM and TAT levels were slightly increased in the TTP/HUS patients who died (Table III). The plasma TM levels of the DIC patients (46.3 ± 24.6 ng/ml) and TTP patients (41.5 ± 28.8 ng/ml) were significantly higher than those of the healthy volunteers (19.8 ± 2.6 ng/ml) ($P < 0.01$). In relation to the prognosis, the plasma TM levels of the DIC patients who died (66.8 ± 18.2 ng/ml) were significantly higher than those of the DIC patients who survived (25.7 ± 7.6 ng/ml) ($P < 0.01$); those of the non-DIC patients who died (52.4 ± 21.1 ng/ml) were significantly higher than those of the non-DIC patients who survived (19.7 ± 5.5 ng/ml) ($P < 0.01$); and those of the TTP/HUS patients who died (85.1 ± 25.7 ng/ml) were significantly higher than those of the TTP/HUS patients who survived (27.7 ± 8.9 ng/ml) ($P < 0.01$). Notably, the plasma TM levels of the non-DIC patients who died were significantly higher than those of the DIC patients who survived ($P < 0.05$) (Fig. 1). The plasma protein C activity was markedly but not significantly reduced in the DIC patients who died (43.2 ± 35.6%), in the non-DIC patients who died (54.7 ± 27.7%), and in the TTP/HUS patients (63.0 ± 23.3%) compared to those who survived (Fig. 2). The plasma antithrombin activity was markedly reduced in the DIC patients who died (61.7 ± 37.1%), in the non-DIC patients who died (77.5 ± 28.6%), and in the TTP/HUS patients (60.9 ± 24.3%) compared to those who survived (Fig. 3). The plasma TF levels were increased in both the DIC patients who died (437 ± 204 pg/ml) and those who survived (397 ± 156 pg/ml), and there was no significant difference in the plasma TF levels between these 2 groups. The plasma TF levels of both the TTP/HUS patients who died (193 ± 36 pg/ml) and those who survived (193 ± 71 pg/ml) were not significantly increased (Fig. 4).

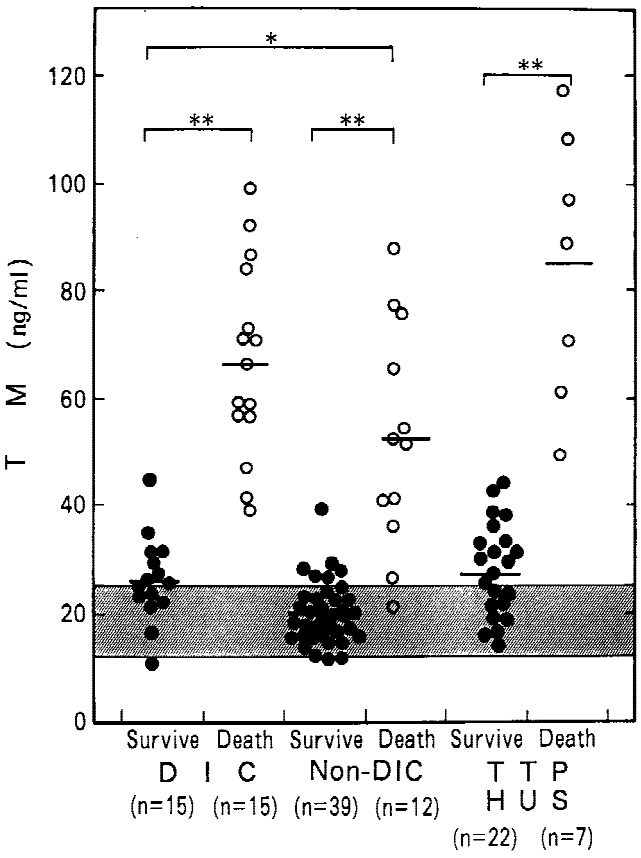

Fig. 1. Plasma thrombomodulin levels in the DIC, non-DIC, and TTP/HUS patients. Survive: the patients who survived more than 4 weeks after the blood sampling. Death: the patients who died within 4 weeks of the blood sampling. *$P < 0.05$, **$P < 0.01$.

DISCUSSION

In this study, although the plasma levels of SFM, TAT, PPIC, D-dimer, and FDP were significantly increased in the patients with DIC, there was no significant difference in these markers between the DIC patients who survived and those who died. These parameters are considered to be sensitive markers for DIC or pre-DIC [13,14], but they were not strongly related to the outcome in the present patient series. PT was slightly prolonged in the DIC patients who died, suggesting that the decreased production

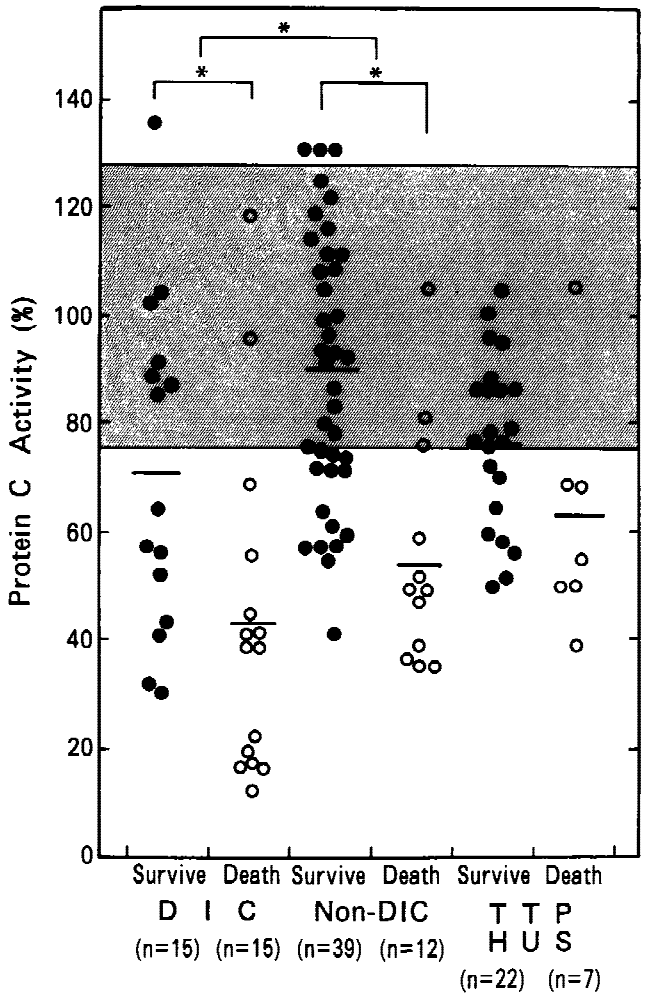

Fig. 2. Plasma protein C activities in the DIC, non-DIC, and TTP/HUS patients. Survive: the patients who survived more than 4 week after the blood sampling. Death: the patients who died within 4 weeks of the blood sampling. *$P < 0.05$.

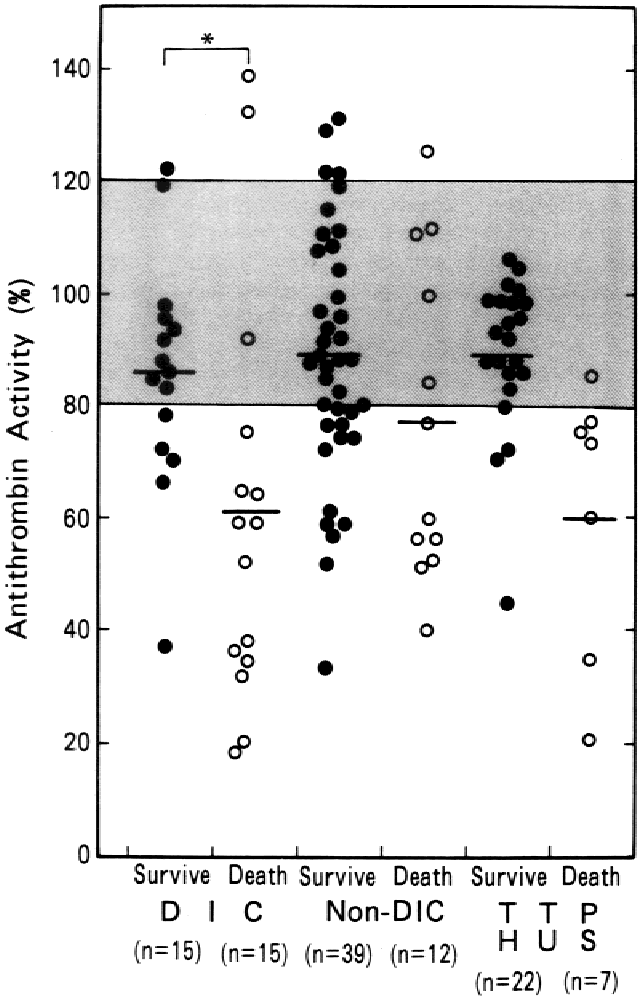

Fig. 3. Plasma antithrombin activities in the DIC, non-DIC, and TTP/HUS patients. Survive: the patients who survived more than 4 weeks after the blood sampling. Death: the patients who died within 4 weeks of the blood sampling. *$P < 0.05$.

ability of coagulation factors in the liver or a severe consumption of coagulation factors might be related to the patient outcome. The plasma levels of SFM, TAT, PPIC, D-dimer, and FDP were also slightly increased in the non-DIC and TTP/HUS patients, but there were no significant differences in these parameters between the non-DIC and TTP/HUS patients who died and those who survived. The hemostatic molecular markers TAT, PPIC, D-dimer, and SFM thus might not be directly related to the outcome.

Increased plasma TM levels were reported in DIC with organ failure [7,8], TTP [8,9], diabetes mellitus with microangiopathy [15], hypercholesterolemia [16], and renal failure [17], and high plasma TM levels were high correlated with organ failure in contrast to other vascular endothelial cell markers, like t-PA, PAI-I, and vWF [9]. As the plasma TM level was significantly increased after hemodialysis [17], it was considered that the plasma TM level was the most specific vascular endothelial cell marker among the hemostatic parameters. Plasma TM levels in DIC and TTP patients were significantly higher than those in the healthy volunteers, suggesting that these patients had severe vascular endothelial cell injuries. The plasma TM levels in the present DIC, non-DIC, and TTP/HUS patients who died were significantly higher than those in the patients who survived. Plasma TM levels are high in patients with renal failure. In the DIC and non-DIC patients, the plasma creatinine levels were all less than 2.0 mg/dl, and these patients did not have severe renal failure. These findings show that TM reflected the patient outcome, and indicate that the outcome of the diseases underlying DIC and TTP/HUS might depend on vascular endothelial cell injuries. Plasma protein C and antithrombin levels were reported to be reduced in sepsis or DIC [18,19], but these levels were also shown to be

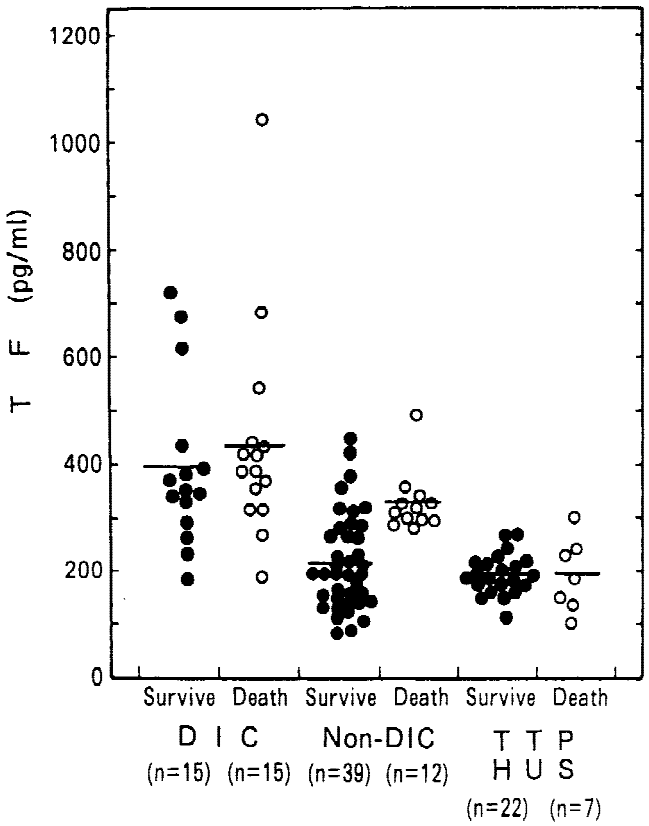

Fig. 4. Plasma tissue factor levels in the DIC, non-DIC, and TTP/HUS patients. Survive: the patients who survived more than 4 weeks after the blood sampling. Death: the patients who died within 4 weeks of the blood sampling.

within normal levels in some patients with DIC [3]. We found that the plasma protein C and antithrombin activities were markedly reduced in the DIC, non-DIC, and TTP/HUS patients who died compared to those who survived. One of the causes of the reduced plasma antithrombin level is thought to be attributable to injured vascular endothelial cells in the patients with organ failure or sepsis [20]. The administration of antithrombin was reported to improve the vascular endothelial injuries in rats treated with endotoxin [21]. We suspect that reduced plasma antithrombin is due not only to consumption but also to the third spaces effects as a result of the increase of permeability in vascular endothelial cells. Our findings suggest that reduced plasma antithrombin and protein C activities are useful markers of systemic vascular endothelial injuries.

Although plasma TF levels were significantly increased in the present DIC patients, there was no significant difference in the plasma TF levels between those who died and those who survived. The plasma TF levels of the TTP/HUS patients who died and of those who survived were not increased. The plasma TF antigen levels were previously observed to increase in DIC patients [22], and TF is expressed in vascular endothelial cells, monocytes, macrophages, neutrophiles, and tumor cells [23,24]. The elevated plasma TF antigens in DIC patients might, therefore, come from stimulated monocytes, macrophages, or neutrophiles.

In conclusion, the outcome of the diseases underlying DIC and TTP is related to vascular endothelial cells, and TM, antithrombin, and protein C are useful markers for systemic vascular endothelial cell injury.

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